# Artificial Gametes Derived from Induced Pluripotent Stem Cells

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## Introduction

The germ cell lineage transmits information from one generation to the following one and gametes represent its ultimate differentiation. Errors in germ cell development can lead to infertility, by which 15% of couples are affected. Artificial gametes are defined as *in vitro*-generated gametes, whose production has potential applications in basic research, disease modeling and clinical use. Induced pluripotent stem cells (iPSCs) provide a platform to generate them.

## Induced pluripotent stem cells

Induced pluripotent stem cells are generated through the transduction of defined transcription factors, such as ***OCT4, SOX2, KLF4****, and* ***C-MYC***, into somatic cells. iPSCs show interesting advantages, but also have some drawbacks:

✓ iPSCs represent an **unlimited source** and there are **not ethical issues** about their use.
✓ **Patient-specific, immune-compatible iPSCs** can be established.
✓ May **allow having genetically-related offspring** to infertile couples.
✗ In contrast, iPSCs **are not identical to ESCs**, retain **epigenetic memory** from the cell type of origin, and show a **diversity of differentiation potentials** among the established iPSCs clones.

## Application of artificial gametes

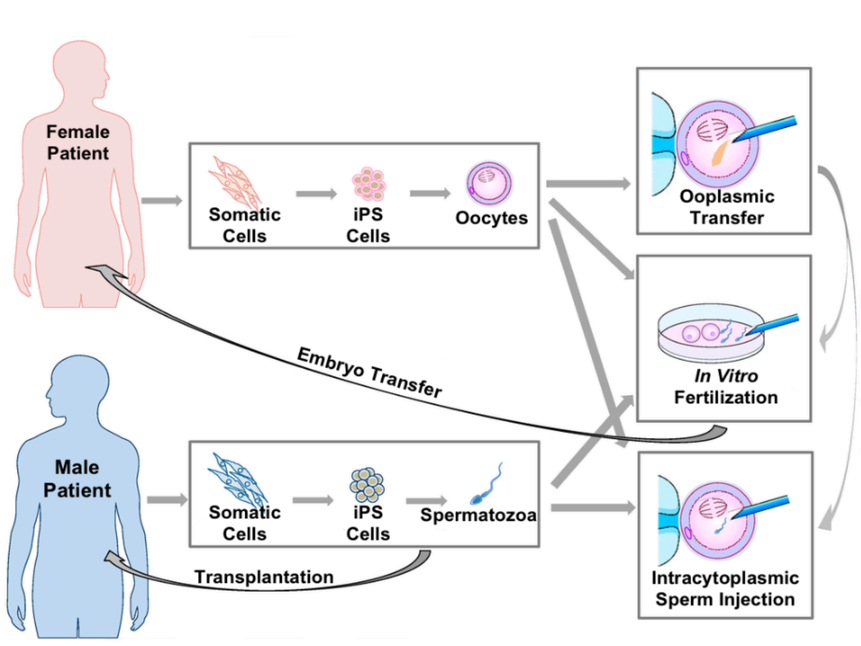


**Figure 1. The potential reproductive uses of iPS cell-based germ cells** (Ishii 2014).

The main application of artificial gametes and iPS cell-derived germ cells is reproduction. iPSCs can be generated from **infertile patients** who do not produce viable gametes. Then iPSCs are differentiated into **gametes**, which can be used for *in vitro* fertilization or intracytoplasmic sperm injection to create **embryos** for transfer.

Other applications of the germ cell induction from iPSCs is **modeling infertility and other anomalies** *in vitro.* Even a **cure of male infertility** by spermatogonial stem cells (SSCs) transplantation may be achieved in the near future. Another application would be **drug testing** on germ cells.

## Objectives

- Define what artificial gametes are and their potential applications.
- Provide an overview about the main strategies used for the differentiation of iPSCs into germ cells *in vitro*, as well as the most important advances that have already been achieved.
- Understand the many barriers that may exist for developing artificial gametes through this approach.

## Methodology

The methodology used has been by searching for information through PubMed (NCBI) and ARE (Àrea de Recursos Electrònics) from UAB. The timeframe in which research reports about the derivation of germ cells from iPSCs have been sought is from 2006 to February 2015. Complementary information has been also consulted when needed.

## Methods*: in vitro* germ cell differentiation from pluripotent stem cells

Methods are divided into **adherent** and **suspension cultures,** which can be performed by embryoid body (EB) formation or co-aggregating with other cells. The efficiency of the process can be improved in the presence of **supplementary factors** and by **overexpressing** germ cell marker genes. Also, pluripotent stem cells can form primordial germ cell-like cells (PGCLCs) through the differentiation of epiblast-like cells (EpiLCs).

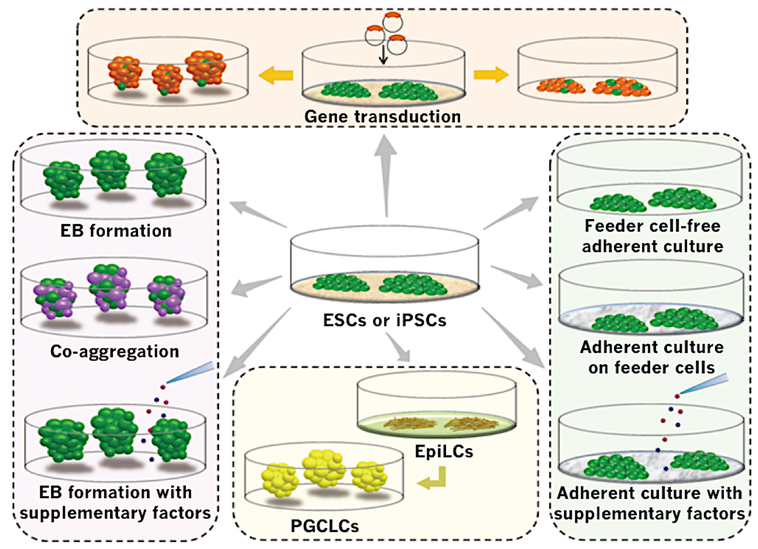


**Figure 2. Summary of methods for the *in vitro* germ cell differentiation of pluripotent stem cells** (adapted from Imamura et al. 2014).

## Research: relevant results

**Table 1. Relevant results of current research about the induction of germ cells from human and mouse induced pluripotent stem cells (iPSCs)** (adapted from Imamura 2014, and Ishii 2014).

| Reference | Specie of iPS cell lines (gender) | Culture methods | *In vitro* derived cells | Evaluation | Remarks |
|---|---|---|---|---|---|
| Hayashi et al. 2011 | **Mouse (XY)** | EpiLCs & PGCLCs induction | **PGCs** | Expression (*Blimp1*, *Stella*, etc.)<br>Genomic imprint (*H19*, *Igf2r*, etc.)<br>Transplantation<br>ICSI | **PGCLCs showed correct function for undergoing spermatogenesis by transplantation into seminiferous tubules of infertile mice and the resultant sperm contributed to offspring** |
| Hayashi et al. 2012 | **Mouse (XX)** | EpiLCs & PGCLCs induction | **PGCs** | Expression (*Blimp1*, *Stella*, etc.)<br>Genomic imprint (*H19*, *Igf2r*, etc.)<br>Transplantation<br>IVF | **PGCLCs could undergo oogenesis when transplanted into mouse ovarian bursa and derived oocytes contributed properly to fertile offspring** |
| Park et al. 2009 | **Human (XY)** | Adherent culture (+human fetal gonadal cells) | **PGCs** | Expression (*VASA*, *cKIT*, etc.)<br>Genomic imprint (*H19*, *PEG1*, etc.) | **PGCs derived from human iPSCs did not initiate imprint erasure as efficiently as those derived from ESCs** |
| Eguizabal et al. 2011 | **Human (XY), (XX)** | Adherent culture (+RA and FRSK, hrLIF, bFGF, CYP26 inhibitor R115866) | **PGCs, spermatids** | Expression (*VASA*, *SCP3*, etc.)<br>Genomic imprint (*H19*)<br>Genome ploidy | **iPSCs of different origin (keratinocytes and cord blood) could be differentiated into haploid cells without the over-expression of germline related transcription factors** |
| Easley et al. 2012 | **Human (XY)** | Adherent culture (mouse SSC culture conditions) | **SSCs, spermatocytes, spermatids** | Expression (*VASA*, *ACROSIN*, etc.)<br>Genomic imprint (*H19*, *IGF2*)<br>Genome ploidy | **Haploid spermatids showed uniparental genomic imprints similar to fertile human sperm on *H19* and *IGF2,* albeit haploid cells from iPSCs showed slightly increased methylation levels on *IGF2*** |
| Ramathal et al. 2014 | **Human (XY)** | Adherent culture (+RA, BMPs, and hrLIF) | **PGCs** | Expression (*VASA*, *STELLA*, etc.) | **iPSCs derived from dermal fibroblasts of males with intact Y chromosome (iAZF) and Y chromosome deletions (iAZFΔ) were used** |

**Abbreviations**: EpiLCs, epiblast-like cells; PGCLCs, primordial germ cell-like cells; RA, retinoic acid; FRSK, Forskolin; hrLIF, human recombinant leukaemia inhibitory factor; bFGF, fibroblast growth factor; PGCs, primordial germ cells; BMPs, bone morphogenetic proteins; SSCs, spermatogonial stem cells; ESCs, embryonic stem cells.

## Conclusions

- iPSCs represent a tool with huge potential in research and clinical applications, but further studies about their epigenetic variations should be done.
- In order to achieve complete functional gametes from iPSCs, it will be required a better understanding about the *in vivo* interaction between germ cells and the *niche* or microenvironment in the gonads.
- The induction of the female germline shows more obstacles than that of the male one.
- Extensive preclinical research will need to be carried out in order to guarantee healthy offspring generated from artificial gametes.

## Selected references

**Easley, C.A. et al.** *Cell Reports*, 2012, 2(3), pp.440–446. **Eguizabal, C. et al.** *Stem Cells*, 2011, 29(8), pp.1186–1195. **Hayashi, K. et al.** *Science*, 2012, 338, pp.971–975. **Hayashi, K. et al.** *Cell*, 2011, 146(4), pp.519–532. **Imamura, M. et al**. *Molecular Reproduction and Development*, 2010, *77*(9), 802–811. **Ishii, T.** *Journal of Clinical Medicine,* 2014, *3*, pp.1064–1083. **Medrano, J.V. et al.** *Stem Cells*, 2012, 30(3), pp.441–451. **Park, T.S. et al.** *Stem Cells*, 2009, 27(4), pp. 783–795. **Polo, J.M. et al.**, *Nature Biotechnology*, 2010, 28(8), pp.848–855. **Ramathal, C. et al.** *Cell Reports*, 2014, 7(4), pp.1284–1297. **Takahashi, K. & Yamanaka, S.** *Cell*, 2006, 126(4), pp.663–676.